#27

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**SJ-0005**
Inventors:	**Danks et al.**
Serial No.:	**09/595,682**
Filing Date:	**June 16, 2000**
Examiner:	**Celine X. Qian**
Group Art Unit:	**1636**
Title:	**Compositions and Methods for Sensitizing and Inhibiting Growth of Human Tumor Cells**

DECLARATION

1. We Mary K. Danks, and Philip M. Potter are co-inventors of the above referenced U.S. Patent Application Serial No. 09/595,682 filed June 16, 2000.

2. We have read and carefully studied the Office Action dated April 9, 2003.

3. Our invention referenced above, teaches a method for sensitizing tumor cells to a chemotherapeutic prodrug comprising transfecting selected tumor cells with a composition comprising an isolated polynucleotide encoding a carboxylesterase (CE). In the Declaration submitted November 18, 2002, we presented *in vivo* evidence that adenovirus is a suitable vector for delivery of CE. We have done further studies showing that human intestinal carboxylesterase (hiCE) can be expressed from two Herpes simplex viral vectors. A human glioma cell line was transduced with the virus, and at various time intervals, the cells were harvested and the levels of CE activity were determined in the extracts. Three different virus vectors were used: rHSVQ1-parent vector; rHSVQ1+hiCEC (rHSVQ1 containing the hiCE cDNA); VQ1+p450+hiCEC (rHSVQ1 containing the rat cytochrome p450 cDNA and the hiCE cDNA). A time dependent increase in CE activity was shown in cells transduced with both vectors containing the hiCE cDNA. Activity peaks were shown on days 4-5, but are expected to continue to increase for extended time periods, see attached Figure 1.

4. Based on this data, the Herpes simplex viral vectors would be an efficient system for delivering CE *in vivo*. Furthermore, other gene delivery systems well known in the art, such as retroviruses, vaccinia viruses, adeno-associated viruses, chemical (polymer or lipid) mediated gene transfer, receptor-mediated DNA uptake, neural stem cell, and physical transfer by gene guns or electroporation, would also be suitable *in vivo* systems for delivering CEs. These systems were taught in the above referenced specification at page 7, lines 6 through 24.

5. Based upon our teaching, *in vivo* gene therapy applications could be performed using various delivery systems and various modes of administration.

6. Further, Figure 2 (attached) shows data that demonstrates a bacterial CE (the pnbA gene from *Bacillus subtilis*) is capable of activating CPT-11. The *B. subtilis* CE produced 52-fold more SN-38 than the control and only about 3 fold less than hiCE. These levels of drug are more than adequate to sensitize mammalian cells to CPT-11. Hence this bacterial CE could be used in enzyme/prodrug therapy approaches with CPT-11.

7. This data demonstrates that a bacterial CE capable of activating CPT-11 was identified using the assays outlined in the specification, see page 30, line 8 through 31, line 3 and also page 35, line 22 through page 36 line 18. Other CEs capable of cleaving prodrugs could be identified by these assays without undue experimentation.

8. As we have taught in the specification, the attached publication by Wadkins et al. further confirms that the rabbit carboxylesterase is capable of cleaving many different types of esters as well as CPT-11 (see Table 2). Another example corroborating our teachings is the attached publication by Wrasidlo et al. which demonstrates a class of prodrugs called epipodophyllotoxins is cleaved by carboxylesterase, in particular a porcine liver carboxylester hydrolase cleaves etoposide (see Table 1). Also attached please find a description of the prodrug Xeloda. This prodrug is a capecitabine derivative that is administered orally and is cleaved in the liver by carboxylesterase to 5-fluorouracil.

9. Computer models are routinely used by those of skill in the art to predict enzyme activity. We have performed studies which confirmed that a computer model used to predict rabbit carboxylesterase's ability to cleave BP-CPT. The model predicted that rabbit carboxylesterase would activate BP-CPT less

efficiently than CPT-11. Our specification teaches the use of a computer model method at page 24, lines 15-24. Growth inhibition and kinetic experiments confirmed that the predictions of the computer model were correct. Our data demonstrated that the predictions obtained from the modeling experiments were validated by both the growth inhibition studies and the kinetic analyses.

10. Prodrugs other than CPT-11 and APC are capable of being effectively cleaved by a carboxylesterase and such drugs may be routinely identified by the methods found in our present application.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Mary K. Danks

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FIGURE 1

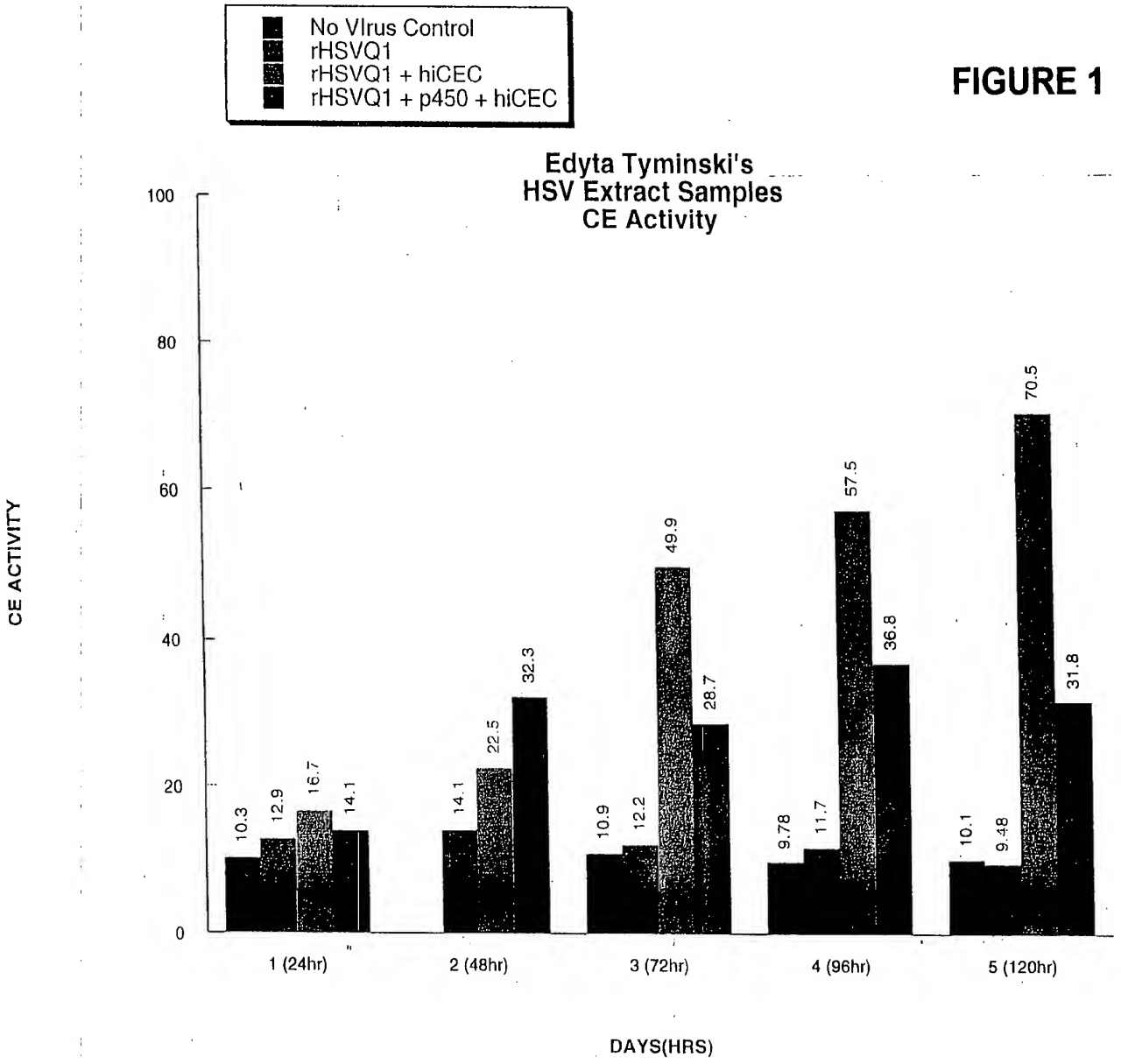

FIGURE 2

Enzyme	0-NPA conversion (μmoles/min/mg)	CPT-11 conversion (pmoles/hr/mg)	Sequence identity to rCE (%)
None	6.7 ± 0.15	3.4	
rCE	2755.5 ± 271.2	2323	100
hCE1	4780.3 ± 279.8	9.6	81
hiCE	1735.6 ± 163.1	654.3	47
BsubCE	1111.9 ± 67.42	177.9	38